Population Dynamics of Citrus Leafminer (Lepidoptera: Gracillariidae) as Measured by Interception Traps and Egg and Larva Sampling in Lime[1]

Jorge E. Peña

University of Florida, Tropical Research and Education Center, 18905 S. W. 280 Street, Homestead, Florida 33031 USA

J. Entomol. Sci. 33(1): 90-96 (January 1998)

Abstract Apical leaf sampling and interception sticky traps were used to assess seasonal density for the citrus leafminer, *Phyllocnistis citrella* Stainton, adults and eggs plus early-instar larvae, respectively, in lime orchards. Traps intercepted greater numbers of adults when placed in the middle or top of the tree canopy than in the lower canopy. Citrus leafminer was most abundant in the spring, summer and fall, and numbers were reduced between November 1994 and January 1995. In 1994, adult capture and egg and larval density were higher than in 1995. The development of an efficient means to estimate citrus leafminer egg density or, alternatively, develop techniques to aid in prioritizing egg sampling was attempted. The probability of finding eggs in the vicinity of a passive interception trap was determined. A linear relationship was found between cumulative number of moths captured and citrus leafminer egg density ($r^2 = 0.52$). A lower variability was recorded for adult traps than for egg densities.

Key Words Citrus leaf miner, *Phyllocnistis citrella,* citrus, adult trapping, egg sampling, sticky traps.

Lime production in Florida is hindered by a number of diseases and arthropod pests including the recently-introduced citrus leafminer, *Phyllocnistis citrella* Stainton (Heppner 1993, Peña and Duncan 1993). The impact of this pest has prompted intensive studies on its biology and population dynamics. Eggs are deposited individually in the adaxial or abaxial sides of young leaves. The eggs hatch in 2 to 10 d depending on the temperature conditions (Knapp et al. 1995). Upon hatching, larvae feed in leaf parenchyma and pupation occurs in the leaf. *Phyllocnistis citrella* is multivoltine in southern Florida, and total generation time can fluctuate between 13 to 52 d depending on temperature (Peña et al. 1996).

Adult leafminer moths are very small (4 mm wingspread) with white and silvery iridescent scales on the forewings. Adults are active from dusk to early morning and apparently oviposit in the evening and early morning. Pheromone traps have been used to trap this insect in China with low efficacy (Tongyuan et al. 1989). Problems probably result from the use of a non-specific sex pheromone (Ando et al. 1985) and from poor understanding of the behavior of the pest.

After the explosive spread of this pest in Florida in 1993 (Heppner 1993), it became necessary to monitor moth flight and egg and larval density to determine population

[1]Received 23 August 1996; accepted for publication 27 December 1996.

peaks of this pest. Current control methods target the damaging late-instar larvae, although recent evidence suggests that damage to lime may be reduced if control of citrus leafminer is aimed at early instars (Knapp et al. 1996). At present, timing of control action is achieved by monitoring damage and larval numbers in the field (Peña 1994). However, adult trap catches may be used to determine population counts of immature stages (Cardé and Elkinton 1984).

The objectives of this study were to: (1) determine the seasonal abundance of citrus leafminer, (2) examine the relative efficiencies of two sampling methods for estimating citrus leafminer densities in limes, and (3) establish if adult catch in traps is correlated with egg and larval population densities.

Materials and Methods

Ten trees were selected randomly from an unsprayed 0.3-ha lime grove at the University of Florida Tropical Research and Education Center, Homestead, FL. Each tree was inspected weekly for eggs from March 1994 through December 1995. Each tree was visually divided into three strata: upper, middle, and lower: ~2.7 to 3.9, 1.4 to 2.5, and 0.2 to 1.3 m from the ground, respectively. On each sampling date, the three apical leaves of new branch terminals were cut from each stratum, totalling 9 samples per tree. Apical leaves have the highest proportion of eggs compared to leaves in the middle and lower part of the flush (Peña and Schaffer 1996). Each leaf was placed in a plastic bag, transported to the laboratory in an ice chest (7 to 10°C), and examined with a dissecting microscope. The number of citrus leafminer eggs and first-instar larvae were recorded per leaf. Means and variances of counts of egg and larvae were calculated for each tree section.

Traps were constructed of transparent plates (23.5 cm diam) coated with Tanglefoot® (The Tanglefoot Company, Grand Rapids, MI). The Tanglefoot was liquified by heating it in a boiler for 1 h at 85°C to facilitate coating the plates. Three traps were placed at the upper, middle and lower tree levels of each tree to determine citrus leafminer moth frequency from March 1994 through November 1995. The effect of north, south, west or east trap orientation was not investigated. Each trap was collected weekly, and the moths counted under a dissecting microscope.

Data analysis. The total number of moths per trap, eggs per leaf, and larval density per leaf were averaged into a weekly mean. Data were subjected to analysis of variance (ANOVA). Means were separated with Tukey's studentized range test ($P = 0.05$) (MEANS TUKEY portion of the General Linear Model Procedure [SAS Institute 1987]). Sampling methods were compared on the assumption that trap catch and egg sampling were the least-biased estimates of insect density. Precision was measured by calculating the coefficient of variation (CV) (CV = 100 × standard deviation/mean) for each sampling date. The effects of temperature (°C) and rainfall (mm) were evaluated by regressing the number of moths trapped per month with the average temperature or rainfall recorded for each month.

Regression analysis and a moth trapped-egg density contingency table were used to examine the relationship between moths captured in sticky traps and egg density. The objective of constructing the contingency tables was to determine the probability of finding some level of egg density per leaf in the vicinity of the sticky trap depending on the capture of a specific number of moths. This was accomplished by categorizing moths captured and egg density or first-instar larval density into broad categories. For each moth and mass density category, the frequency and percentage of frequency in each category was determined. The moth categories used in this analysis consisted of 0 to 0.89, 0.9 to 2.0, 2.1 to 5, 5.1 to 8.0, 8.1 to 20, 21 to 50, and >50 moths. Egg density categories were 0 to 0.22, 0.23 to 0.50, 0.51 to 1.0, 1.1 to 3.0 and 3.1 to 5.0 eggs per leaf. First-instar larval density categories were 0 to 0.09, 0.1 to 0.22, 0.23 to 0.5, 0.51 to 1, 1.1 to 3 per leaf. The regression analysis and moth catch-egg mass contingency table were computed using SAS, Prog GLM, and Proc Freq, respectively (SAS Institute 1987).

Results and Discussion

Egg and first-instar larvae. Mean egg and first-instar larval counts from each tree canopy position provided information on the reliability of canopy position as a relevant sampling location. The number of eggs per leaf was similar for each canopy position. A significantly higher number of first instar-larvae was found in the middle canopy as opposed to the upper and lower canopies (Table 1).

Adult sampling. More adults were captured in traps at 2.70 and 1.40 than at 0.20 m from the ground (Table 1). This result was expected because of the high counts observed in traps located in the upper canopy during spring 1994 and the high relative counts recorded in the middle canopy from March 1994 to November 1995 (Fig. 1).

Seasonal abundance. The seasonal abundance of moths, as collected by interception traps, and egg and early larval density is shown in Fig. 2. Citrus leafminer adults occurred through the year. The density of moths trapped during 1994 was 2.5 times higher than in 1995. This reduction in infestation numbers may be the result of significant parasitism caused by the native parasitoids that attacked the leafminer since its arrival in Florida (Peña et al. 1996). The number of moths trapped (y) were not influenced by the amount of rainfall (x) ($r^2 = 0.003$; $F = 0.06$; $P > 0.81$; $y = 9.29 + 0.005x$) but might have been influenced by temperature ($r^2 = 0.25$; $F = 6.62$; $P > 0.02$; $y = -33.78 + 1.73x$). Peak numbers of adults were trapped in May, July, October 1994 and April, July, August, November 1995 (Fig. 2). During the December 1994 through March 1995 period, population levels were below 0.5 moths per trap. Influence of warm temperatures on catches was observed in the early spring season. For example, night-time temperatures during April and May were warmer than during the previous months, and both the number of total moths caught and the

Table 1. Mean number of citrus leafminer adults and distribution of citrus leafminer eggs and first-instar larvae at different heights on the lime tree canopy

Trap height (m)	Position within the canopy	No. moths/ trap/week Mean ± SE	Eggs/ leaf Mean ± SE	First-instar/ leaf Mean ± SE
2.70-3.90	Top	10.07 ± 1.71a	0.74 ± 0.05a	0.46 ± 0.03b
1.40-2.60	Middle	9.33 ± 1.08a	0.76 ± 0.05a	0.64 ± 0.05a
0.20-1.30	Bottom	4.68 ± 0.48b	0.75 ± 0.05a	0.51 ± 0.04b

Means within column followed by the same letter are not significantly different ($P = 0.05$; Tukey's studentized range test [SAS Institute 1987]).

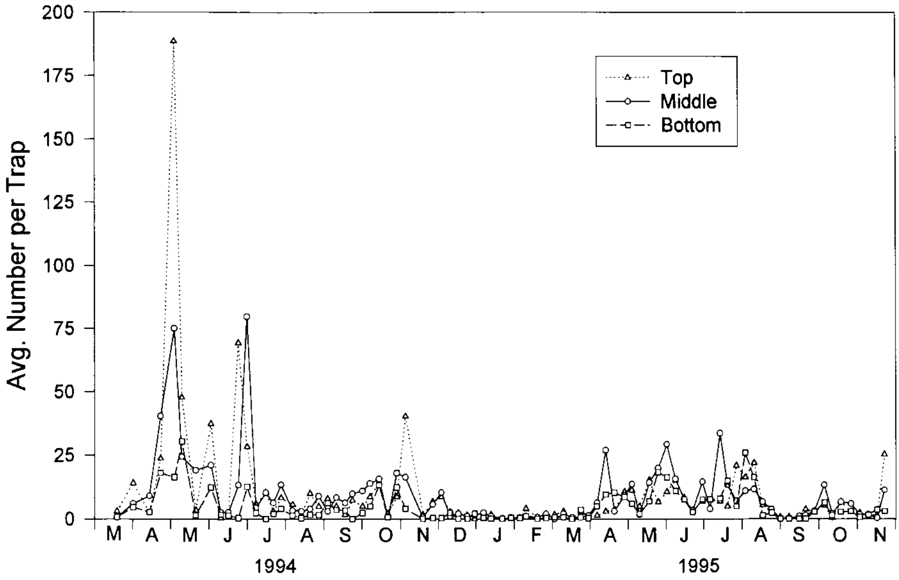

Fig. 1. Within-tree capture of citrus leafminer moths per week of three different heights from the soil from March 1994 through November 1995.

number of eggs and early-instar larvae increased sharply. Several annual peaks of moth abundance corroborates that citrus leafminer is multivoltine. Eggs and early-instar larvae were exceptionally high in 1994 compared to 1995. Egg and larval population peaks were observed in spring, summer and early fall, and levels were drastically reduced during winter (Fig. 2).

Comparison of techniques. The mean CV of weekly samples of citrus leafminer was 139 ± 73 for egg samples, 169 ± 68 for larval samples, and 107 ± 31 for adults in traps. Seasonal trends of CV for the egg and adult sampling methods were significantly correlated (r^2 = 0.43), and CV's from the two methods differed significantly (Wilcoxon matched pairs signed-rank test; t = 2.56, SE 12.26). According to these results, a lower variability was recorded for adult traps. CV's are conventionally used as measures of variability of sampling techniques (Ruesink 1980). Two ways of reducing CV's are to develop more effective sampling techniques and to increase the number of samples taken (Karandinos 1976).

Correlation between population density and adult catch. Regression analysis indicated that a linear relationship exists between pooled number of moths per trap per week and egg density per leaf per week (P < 0.0006), but moths per trap (x) explained little of the variation in egg density (y) (r^2 = 0.14). The regression equation was as follows; (y = 0.56 + 0.0281x). A better correlation (r^2 = 0.52; P < 0.0001) was observed between cumulative catch for moths trapped during 1994 and 1995 and cumulative egg density for a series of two-month periods. Cumulative catches for every sampling period were highly correlated with cumulative egg populations (r^2 = 0.78 to r^2 = 0.98) (Table 2). Cumulative data from the moth catch-egg density table indicates there is a relationship between different categories of moths captured

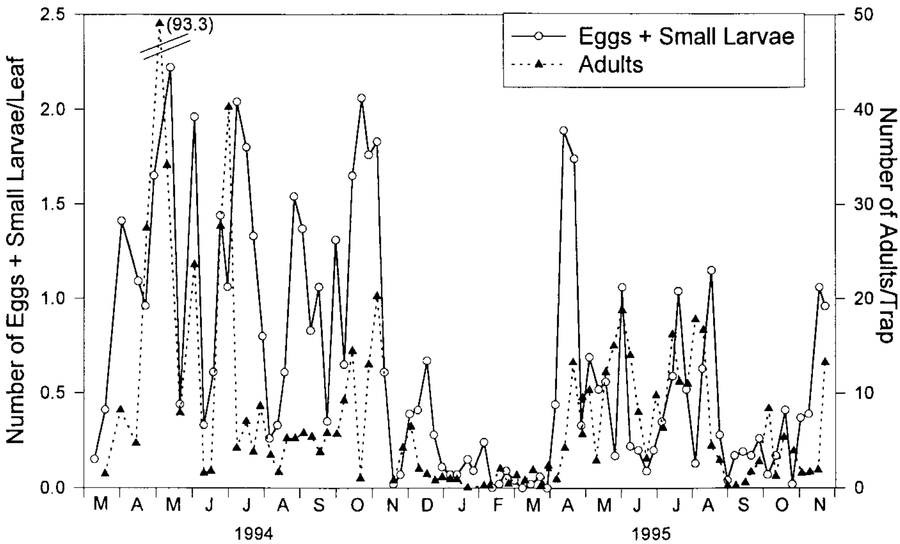

Fig. 2. Dynamics of citrus leafminer adult moth trapping and egg and first larval density from 1994 through 1995.

Table 2. Relationship between moth catch in interception traps and cumulative egg density at different sampling periods between 1994 and 1995.

Sampling period	b_0 intercept	b_1 moth	r^2
April-May 1994	1.26	0.04 (0.004)	0.91
June-July	−0.63	0.10 (0.01)	0.82
August-September	0.73	0.15 (0.012)	0.96
October-November	2.32	0.10 (0.005)	0.98
December-January 1995	0.25	0.15 (0.028)	0.83
February-March	3.01	3.57 (0.715)	0.78
April-May	−17.35	28.71 (1.964)	0.96
June-July	−1.06	23.57 (2.84)	0.91
August-September	−3.54	16.07 (1.73)	0.92
Common	1.36	0.04 (0.005)	0.52

$y = b_0 + b_1x$; y = cummulative egg density; x = moths trapped per week; All parameters are significant ($P >$ 0.05; Proc Reg [SAS Institute 1987]). Numbers in parentheses are SE of estimates.

Table 3. Probability (P) the egg density per leaf in the location of the trap will be of a specified level based on the capture of certain number of moths (moths/trap) (n is the number of traps in each category).

Egg density	Moths per trap													
	0-1	n	1-2	n	2-5	n	5-8	n	8-20	n	21-50	n	>50	n
0.0-0.2	0.88	135	0.42	45	0.18	18	0.20	18	0.20	36	0.0	0	0.00	0
0.2-0.5	0.12	18	0.58	63	0.09	9	0.50	45	0.15	27	0.2	9	0.00	0
0.5-1.0	0.00	0	0.00	0	0.18	18	0.10	9	0.30	54	0.0	0	0.00	0
1.0-3.0	0.00	0	0.00	0	0.36	36	0.20	18	0.30	54	0.8	36	1.00	9
3.0-5.0	0.00	0	0.00	0	0.00	0	0.00	0	0.05	20	0.0	0	0.00	0

and egg density (Table 3). For example, if a trap captures 0 to 1 moth, the probability of finding an egg density of 0 to 0.2 is 0.88; if >5 moths are captured, the probability of finding an egg density of >0.2 is 0.50. If more than 21 moths are captured, the probability of finding an egg density >1 is 0.80.

In the same way, regression analysis indicated that a significant linear relationship exists between moths per trap (x) and first-instar larval density (y) ($P = 0.0027$), but again, moths per trap explained little of the variation in larval density ($r^2 = 0.11$). The regression equation was: $y = 0.39 + 0.016x$. The moth catch-first-instar density indicates there is a weaker relationship between categories of moths captured and larval density than from moths and egg density (Table 4). For example, if a trap captures <0 to 0.88 moths the probability of finding a larval density of 0 to 0.09 is 0.71, if >5 moths are captured the probability of finding an egg density of 1 is only 0.40.

If both factors, egg density and first-instar, are added, the regression analysis indicated that a significant linear relationship exists between moths per trap and the two added factors ($P < 0.0001$), but again moth density per trap explained little of the variation on egg and larval density ($r^2 = 0.17$). The use of a contingency table for both the egg density and the larval density does not improve the relationship between

Table 4. Probability (P) of first-instar larvae density per leaf in the location of the trap will be of a specified level based on the capture of certain number of moths (moths/trap). (n is the number of traps in each category).

Larval density	Moths per trap													
	0-1	n	1-2	n	2-5	n	5-8	n	8-20	n	21-50	n	>50	n
0.0-0.1	0.71	90	0.18	27	0.21	36	0.00	0	0.16	33	0.00	0	0.00	0
0.1-0.2	0.29	36	0.25	36	0.16	27	0.20	18	0.11	18	0.20	9	0.00	0
0.2-0.5	0.00	0	0.37	54	0.11	18	0.20	18	0.26	45	0.00	0	0.00	0
0.5-1.0	0.00	0	0.13	18	0.32	54	0.40	36	0.37	63	0.40	18	1.00	9
1.0-3.0	0.00	0	0.06	9	0.21	36	0.20	18	0.11	18	0.40	18	0.00	0

different categories of moths captured and egg and larval density. For example, if a trap captures 0.88 moths per trap, the probability to have an egg and larval infestation of 0.09 is only 0.46.

These results indicate it is possible to determine the probability of finding a specified egg density in the vicinity of a trap depending on the capture of a specific number of moths per trap. These probabilities tended to change in a predictable manner, with the capture of more moths resulting in a higher probability of finding a greater egg density. Therefore, within the categories reported in this paper, the number of moths captured per trap is related to egg density. However, variability in catches may be tied to population density in the surrounding orchards and may represent the potential of moths for immigration.

Acknowledgment

I thank R. Duncan, S. Sundhari and A. Trimiño for assistance and R. M. Giblin-Davis, R. Bullock, C. McCoy and B. Schaffer for critically reviewing the manuscript. This research was partially supported by the Florida Citrus Production Research Advisory Council. This is Journal Experimental Series No. R-05285.

References Cited

Ando, T., K. Y. Taguchi, M. Uchiyama, T. Ujiye and H. Kuroko. 1985. (7Z-11Z)-7,11–hexadecadienal sex attractant of the citrus leafminer moth. *Phyllocnistis citrella* Stainton (Lepidoptera: Phyllocnistidae). Agric. Biol. Chem, Tokyo 49: 3633-3653.

Cardé, R. T. and J. S. Elkinton. 1984. Field trapping with attractants: methods and interpretation, Pp. 111-129. *In* H. E. Hummel and T. A. Miller [eds.], Techniques in Pheromone research. Springer-Verlag, New York.

Heppner, J. 1993. Citrus leafminer, *Phyllocnistis citrella,* in Florida. Trop. Lep. 4: 49-64.

Karandinos, M. 1976. Optimal sample size and comments on some published formulae. Bull. Entomol. Soc. Am. 22: 417-421.

Knapp, J., L. Albrigo, H. Browning, R. Bullock, J. Heppner, D. Hall, M. Hoy, R. Nguyen, J. E. Peña and P. A. Stansly. 1995. Citrus leafminer, *Phyllocnistis citrella* Stainton: Current status in Florida-1995. University of Florida, Florida Cooperative Extension Service, Gainesville. 35 p.

Knapp, J., H. Browning, L. G. Albrigo, J. E. Peña, P. Stansly and R. C. Bullock. 1996. Management of the citrus leafminer: chemical options. Citrus Industry, March 1996.

Peña, J. E. 1994. Update on control of the citrus leafminer. Proc. Fla. State Hort. Soc. 107: 72-75.

Peña, J. E. and R. E. Duncan. 1993. Control of citrus leafminer in south Florida. Proc. Fla. State Hort. Soc. 106: 47-51.

Peña, J. E., R. Duncan and H. W. Browning. 1996. Temporal distribution of *Phyllocnistis citrella* (Lepidoptera: Gracillariidae) and its parasitoids in south Florida citrus. Environ. Entomol. 25: 698-702.

Peña, J. E. and B. Schaffer. 1997. Intraplant distribution and sampling of the citrus leafminer (Lepidoptera: Gracillariidae) on lime. J. Econ. Entomol. 90: 458-464.

Ruesink, W. 1980. Introduction to sampling theory, Pp. 61-78. *In* M. Kogan and D. C. Herzog [eds.], Sampling methods in soybean entomology. Springer Verlag, New York.

SAS Institute. 1987. SAS/STAT user's guide, release 6.03 ed. SAS Institute, Cary, NC.

Tongyuen, D., X. Jinjun, W. Zehua and K. Fanlei. 1989. 7,Z,7,11-hexadecadienal: sex attractant of *Phyllocnistis wempella* Liu et Zeng. Kunchong Zhishi. 3: 147-149.